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ORPHAN-INDUCED LACTATION IN *TURSIOPS* AND ANALYSIS OF COLLECTED MILK

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ABSTRACT

Two non-lactating, non-gravid female bottlenose dolphins, *Tursiops truncatus*, (SLA, age 32 yr and TOD, age 34 yr) began lactating within one week after being housed with a 6.5- and 6.75-month-old orphaned calf, respectively. Cooperative swimming and nursing behaviors were quickly evident in both cow/calf pairs. During the first 2.5 mo a milk substitute, eventually in combination with some whole fish, was used to supplement nursing in both calves. After this initial period, supplemental feeding was discontinued. SLA then became her adopted calf's sole source of nutrition for the next 5 mo, followed by another 6 mo during which the calf shifted to a predominantly fish diet with occasional nursing. TOD's orphan also continued to nurse beyond the 2.5-mo supplementation period and began voluntarily accepting small quantities of fish; suckling continued over the next year while fish consumption increased. Using a modified human breast pump, trainers collected milk at intervals from the adult dolphins throughout the lactation period. The first milk collected from TOD (day 8 after housing with calf) and SLA (day 12 with her calf) contained 6.0% and 10.3% fat, respectively. On day 68 TOD's milk fat had increased to 23.5%, and on day 37 that of SLA measured 22.5%. These later values are similar to those reported for normally lactating bottlenose dolphins.

Key words: bottlenose dolphin, *Tursiops truncatus*, milk, lactation, relactation, induced lactation, nursing, orphan.

Non-parturient, orphan-induced lactation has been reported in women with or without previous childbearing experience; often they are relatives of the nurtured orphan (Waletzsky and Herman 1976, Auerbach 1981, Auerbach and Avery 1981, Ryba and Ryba 1984). The terms relactation and induced lactation have been used to further define these events. Relactation describes the condition in which a woman who has been pregnant and has previously lactated, but has been dry for an extended period of time, begins milk production without first maintaining pregnancy. Induced lactation designates milk production in the absence of previous pregnancy or lactation. Richardson (1975) describes three cases of relactation even in postmenopausal grandmothers. In the most extreme instance a 55-yr-old woman, who had last given birth 32 yr previously, relactated and successfully reared her daughter's orphaned neonate. Relactation and induced lactation have also been reported in other species, including goats, cattle (Richardson 1975), horses (Anonymous 1973), and sheep (Kendrick *et al.* 1992). Here we describe such events in two Atlantic bottlenose dolphins, *Tursiops truncatus*.

Materials and Methods

Dolphins—The principal dolphins in this study were SLA (age 32 yr, length 261 cm, weight 202 kg) and TOD (age 34 yr, length 269 cm, weight 205 kg). As part of routine veterinary care each animal received biannual physical examinations, which included blood samples collected during volunteer tail fluke presentations. Serum analysis in 1978 for progesterone (Harrison and Ridgway 1971, Kirby and Ridgway 1984) revealed that SLA was pregnant. She calved in 1979 and nursed her calf until it was weaned in 1981. SLA is a grandmother dolphin since her calf (SAY) now has her own calf (ARI); prior to the relactation event, SLA shared the same pen complex but was not physically housed with SAY and ARI. SLA has not tested positive for pregnancy and no lactation was observed from 1981 to 1992. TOD has lived at our facilities for 24 yr, during which time she has neither been pregnant nor has lactated until her lactation event in 1992. The orphaned dolphins were 2 males, aged 6.5 and 6.75 mo when introduced to SLA and TOD, respectively. For comparison with the milks of SLA and TOD, milk samples were collected from 4 other *Tursiops*, including ELL (age 16 yr), SAY (age 12 yr) and COR (age 7 yr) (each nursing her own offspring) and PNN (age 12 yr) whose calf was stillborn. Seven samples each were collected from ELL and SAY on a series of lactational days after several months of nursing. As a measure of milk fat and protein during early lactation in dolphins nursing their own calves, a sample was collected from COR on lactational day 8 and from PNN during her first day of lactation.

Diet—Each dolphin received a varied daily fish diet (stored as fresh frozen), including herring, *Clupea pallasi* or *Clupea harengus*; Pacific mackerel, *Scomber japonicus*; capelin, *Mallotus villosus*; squid, *Loligo opalescens*; and Columbia river smelt, *Thaleichthys pacificus*. Although small fish could swim through the netted enclosures housing the dolphins in San Diego Bay (32°42′N, 117°14′W), these fish were not considered a significant contribution to the dolphins' diet.

Lactation and milk collection—Shortly after introduction to their respective orphans, both SLA and TOD began lactating. A modified human breast pump, which we had developed earlier for milk collections on other dolphins, was used to sample milk for nutrient analysis. The device consisted of a hand-operated vacuum pump, a respirator mask trimmed to conform to the dolphin's body, and an in-line collection vial (Fig. 1). The mask was pliable enough to permit

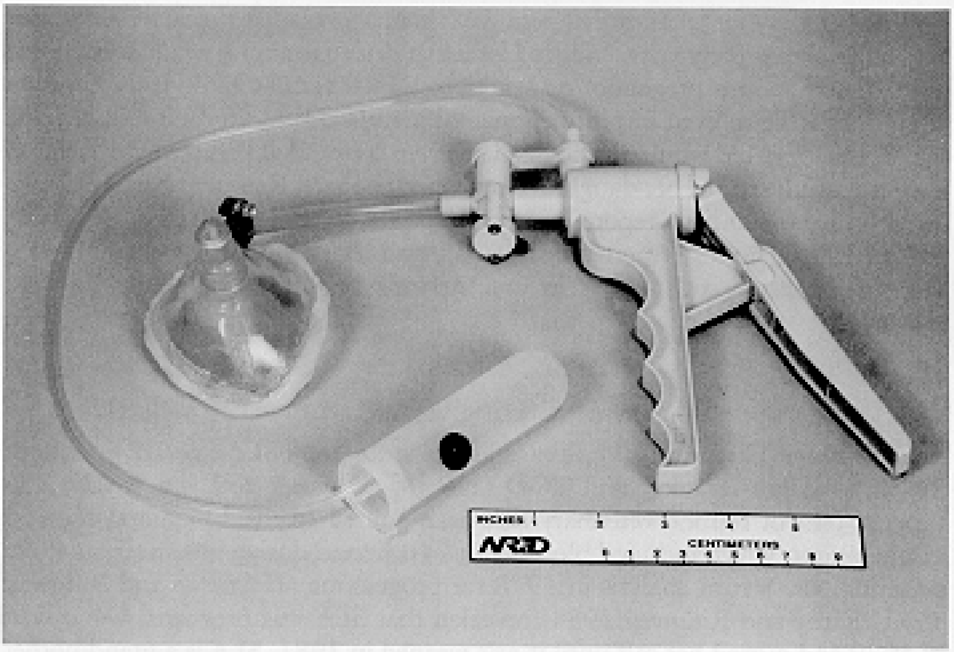

Figure 1. The dolphin milking device is a closed system consisting of a hand-operated vacuum pump, suction mask, and collection vial. All components are linked by plastic tubing.

an effective seal, yet sufficiently thick to resist collapse under vacuum. As U.S. Navy working dolphins for more than 20 yr, SLA and TOD were accustomed to husbandry training and soon adapted to the milking process. Both dolphins were conditioned to present ventrally at the side of their respective enclosures in a position that would facilitate milk collection. They were then acclimated to allow one trainer to support the peduncle just enough to keep the mammary slits above the water surface. Meanwhile, a second trainer massaged the region of the mammary slit to simulate the prenursing stimulus and encourage milk let-down. The mammary region was then rinsed with distilled water and the milk collection device was positioned over one mammary slit; suction was applied and the milk sample was withdrawn. It appeared to us that once the dolphins felt the suction, the release of milk was almost immediate; however, amounts of milk collected at any one milking varied, ranging from 1 to 44 ml. Where individual milkings were too small for analysis, pooled samples from the same or different days were prepared prior to submission to the laboratory (Table 1).

Analytic methods—Milk samples were apportioned in 4-ml vials and stored at −70°C until sent to the laboratory[1] for analysis. Protein was determined by converting all organic nitrogen (N) in the sample to ammonia by sulfuric acid digestion; nitrogen percentage was determined and converted to protein using the formula: protein = N × 6.38 (Official Methods of Analysis [AOAC] 1990,

[1] Hazleton Wisconsin Laboratories, 3301 Kinsman Blvd., Madison, Wisconsin 53707.

Table 1. Milk fat and protein concentrations in 6 bottlenose dolphins, *Tursiops truncatus,* during various stages of lactation.[a]

Dolphin	Lactation day[b]	% Fat	% Protein	Sample size
TOD	8	6.0	11.6	4 ml
	11, 12, 18	7.7	14.0	60 ml
	19	8.4	15.0	5 ml
	35	13.6	12.4	10 ml
	68, 71	23.5	13.3	64 ml
	127, 138	26.5	12.7	60 ml
SLA	10	10.3	8.4	8 ml
	22	5.7	4.7	7 ml
	37	22.5	10.9	8 ml
	79, 82, 93, 96	20.0	9.7	60 ml
	114	10.0	7.4	7 ml
ELL	169–180	20.4	9.8	62 ml
SAY	94–145	21.9	9.8	61 ml
COR	8	19.3	14.1	4 ml
PNN[c]	1	20.7	18.3	4 ml

[a] TOD and SLA were induced to lactate by orphan calves; ELL and SAY were nursing their own offspring; COR's calf died 3 d prior to milk sampling; PNN's calf was stillborn.

[b] Where multiple lactation days are listed, samples from each day indicated were pooled for a single analysis. For both ELL and SAY, 7 d of single samples were combined within the interval of days indicated for a single analysis. (Larger samples than needed for fat and protein analysis were pooled in some instances to support other analytic work being conducted at the same time.)

[c] PNN was never suckled since her calf was stillborn; her single milk sample was collected at 21 h after the calf's delivery.

15th Edition, Methods 955, 040, and 979.09, Association of Official Analytical Chemists, Arlington, Virginia, modified). Fat was hydrolyzed on a water bath using hydrochloric acid. The fat was extracted from milk samples using ether and hexanes. The extract was then dried and weighed (AOAC, 1990, Method 922.06, 954.02, modified).

Results

Both SLA and TOD were immediately attentive to their respective calves. Each physically investigated her calf, repeatedly nuzzling the calf's genital area, and assumed typical cow/calf swimming behaviors with the calf positioned below or directly to the side of the older dolphin. Each calf immediately began nursing attempts, despite several feedings per day of a milk substitute. The adults appeared to encourage suckling behaviors by assuming gliding postures which facilitated the calf's access to the mammary region. Nursing attempts were frequent, as many as 10 per hour.

SLA (age 32 yr) had been dry and non-pregnant for 11 yr when introduced to the 46 kg, 6.5-mo-old orphan calf in October 1992. We reasoned that SLA's previous experience in calf rearing might make her an effective companion to the orphan. Since we did not expect relactation following introduction to the calf and because the calf was not yet eating fish, a regimen of formula feeding by stomach tube was begun. Despite several regular feedings daily, the orphan persistently attempted to nurse SLA. Since nursing behaviors continued, we began to consider the possibility that SLA could be producing milk. On the 10th day following SLA's introduction to the orphaned calf, a trainer noted possible milk secretion into the water. Examination confirmed that the mammary region was somewhat swollen and that SLA was lactating. A milk sample was successfully collected for analysis during this initial examination. Several supplemental feedings per day with a milk substitute and, eventually, some whole fish were provided to the calf, but were gradually withdrawn after 2.5 mo. SLA then became the sole source of nutrition for the growing orphan for the next 5 mo, after which the calf began voluntarily taking fish (age 15.5 mo); suckling continued for another 6 mo, although nursing frequency declined as fish consumption increased. Weight gains by the calf continued for over a month following termination of supplemental feeding and then declined from 12 to 14 mo of age (Fig. 2a). Subsequent weighing at 19.5 mo of age demonstrated recovery in the growth curve, 4 mo after accepting fish voluntarily.

The second dolphin (TOD, age 34 yr) had never been pregnant nor had she lactated in more than 24 yr in our care before being placed with a 73 kg, 6.75-mo-old orphan calf. Nursing behaviors by the calf and apparent swelling in TOD's mammary region prompted an examination which confirmed lactation on day 5 following calf introduction. A milk sample was successfully collected on day 8. Nursing behaviors were evident between cow and calf during the ensuing 2.5 mo, despite supplementation with a milk substitute and/or whole fish which was continued until the calf voluntarily accepted fish at the end of the 2.5-mo period. The orphan continued to suckle TOD while increasing his fish consumption over the next year. During this entire period, following an initial interval at the start of supplementation when body weight declined, weight gains continued (Fig. 2b). There was never a time for this calf when nutritive intake depended entirely on nursing, since he began to accept fish voluntarily as soon as supplementation was discontinued.

Table 1 provides a comparison of the protein and fat content in milk from TOD and SLA, with that of ELL and SAY, two dolphins nursing their own calves. A comparison is also made with COR on lactational day 8, following loss of her calf 3 d earlier, and with that of PNN, whose calf was stillborn, on her first day of lactation. TOD's milk showed an increase in fat content from

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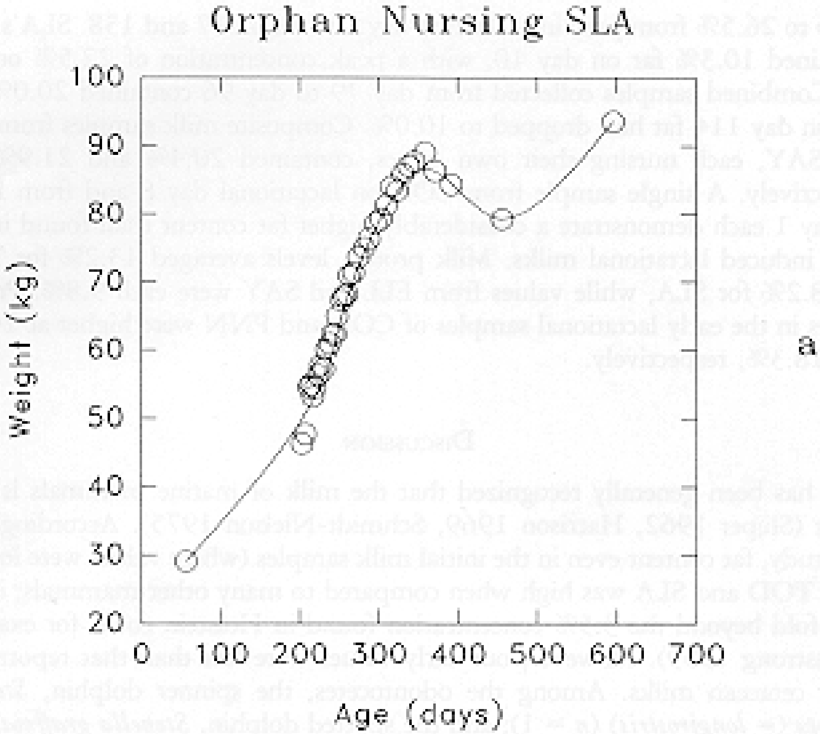
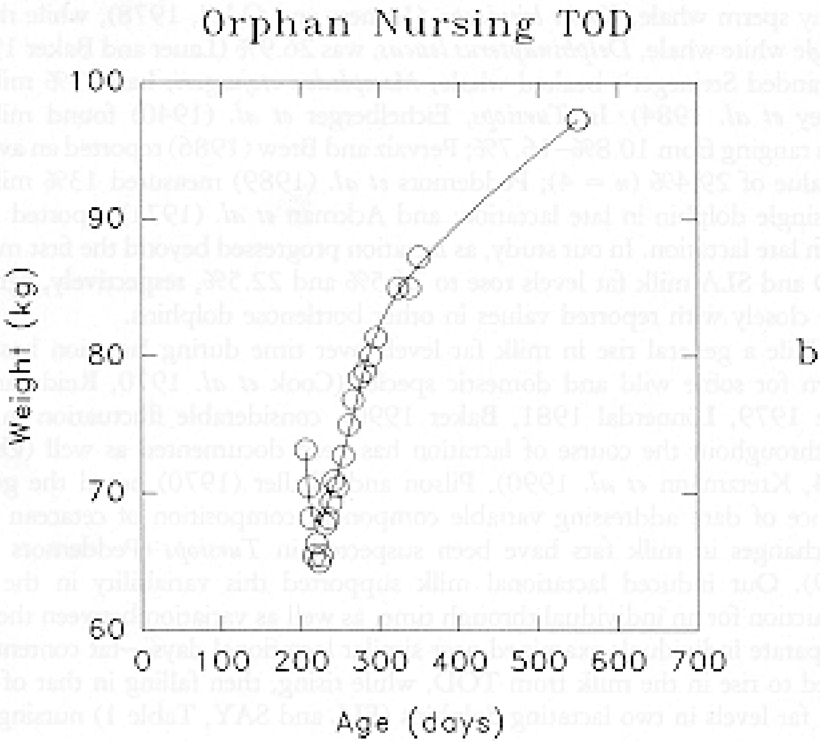
Figure 2. Weight gains by orphaned calves. a. The calf nursing SLA showed consistent weight gains throughout the supplemental period and during the initial interval where nutritional intake was limited to suckling. This was followed by a decline in weight, with growth-curve recovery after 4 mo on voluntarily accepted whole fish. b. The calf nursing TOD declined in weight initially, prompting an increase in supplemental feeding until a positive growth curve was achieved. Weight gains were consistent thereafter with the calf switching immediately to voluntary fish intake when supplemental feedings were discontinued.


Orphan Nursing SLA
100
90
80
70
60
50
40
30
20
Weight (kg)
0 100 200 300 400 500 600 700
Age (days)
a


Orphan Nursing TOD
100
90
80
70
60
Weight (kg)
0 100 200 300 400 500 600 700
Age (days)
b

6.0% to 26.5% from post-introduction day 8 to days 127 and 138. SLA's milk contained 10.3% fat on day 10, with a peak concentration of 22.5% on day 37. Combined samples collected from day 79 to day 96 contained 20.0% fat, but on day 114 fat had dropped to 10.0%. Composite milk samples from ELL and SAY, each nursing their own calves, contained 20.4% and 21.9% fat, respectively. A single sample from COR on lactational day 8 and from PNN on day 1 each demonstrate a considerably higher fat content than found in our early induced lactational milks. Milk protein levels averaged 13.2% for TOD and 8.2% for SLA, while values from ELL and SAY were each 9.8%. Protein values in the early lactational samples of COR and PNN were higher at 14.1% and 18.3%, respectively.

Discussion

It has been generally recognized that the milk of marine mammals is high in fat (Slijper 1962, Harrison 1969, Schmidt-Nielson 1975). Accordingly, in our study, fat content even in the initial milk samples (where values were lowest) from TOD and SLA was high when compared to many other mammals; it was 2–3 fold beyond the 3.5% concentration found in Holstein cows, for example (Armstrong 1959). However, our early values were less than that reported in other cetacean milks. Among the odontocetes, the spinner dolphin, *Stenella microps* (= *longirostris*) ($n = 1$); and the spotted dolphin, *Stenella graffmani* (= *attenuata*) ($n = 8$), Pilson and Waller (1970) presented milk-fat levels of 26.2% and 25.3% (range 17%–32%), respectively. Milk fat was 15.3% in a stranded pygmy sperm whale, *Kogia breviceps*, (Jenness and Odell, 1978), while that of a single white whale, *Delphinapterus leucas*, was 26.9% (Lauer and Baker 1969). A stranded Stejneger's beaked whale, *Mesoplodon stejnegeri*, had 17% milk fat (Ullrey *et al.* 1984). In *Tursiops*, Eichelberger *et al.* (1940) found milk-fat levels ranging from 10.8%–16.7%; Pervaiz and Brew (1986) reported an average fat value of 29.4% ($n = 4$); Peddemors *et al.* (1989) measured 13% milk fat in a single dolphin in late lactation; and Ackman *et al.* (1971) reported 19%, also in late lactation. In our study, as lactation progressed beyond the first month, TOD and SLA milk fat levels rose to 26.5% and 22.5%, respectively, agreeing more closely with reported values in other bottlenose dolphins.

While a general rise in milk fat levels over time during lactation has been shown for some wild and domestic species (Cook *et al.* 1970, Reidman and Ortiz 1979, Lönnerdal 1981, Baker 1990), considerable fluctuation in milk fats throughout the course of lactation has been documented as well (Øftedal 1984, Kretzmann *et al.* 1990). Pilson and Waller (1970) noted the general absence of data addressing variable component composition of cetacean milk, but changes in milk fats have been suspected in *Tursiops* (Peddemors *et al.* 1989). Our induced lactational milk supported this variability in the milk production for an individual through time, as well as variation between the milk of separate individuals examined near similar lactational days—fat content continued to rise in the milk from TOD, while rising, then falling in that of SLA. Milk fat levels in two lactating dolphins (ELL and SAY, Table 1) nursing their own calves at our facility were comparable to the peak value in SLA but were actually exceeded by TOD's later samples. Protein values were also similar to ranges previously reported in the bottlenose dolphin (Eichelberger *et al.* 1940, Pervaiz and Brew 1986).

Our observations demonstrate that dry adult dolphin females can be brought into lactation by repeated nursing attempts from unrelated calves. Kastelein *et al.* (1990) reported growth in a *Tursiops* calf which suckled a foster mother for 31 d until the calf died from complications of wounds inflicted by its original mother. However, the foster mother in this case was already lactating, having lost her own nursing calf 8 d earlier. SLA's experience can be described as relactational since she had been previously pregnant with subsequent lactation and suckling of her calf. Primary induced lactation may describe TOD's circumstance since she had no pregnancy or associated milk production in the 24 yr in our care; however, we must allow the possibility that she had lactated as a young adult since she was introduced to our facility at an estimated age of 10 yr, just beyond the generally accepted age of 7 yr for attainment of sexual maturity in the female bottlenose dolphin.

The capacity of induced lactational milk to provide total nutritional support to growing dolphin calves remains undetermined from our observations. The two dolphins (COR and PNN), producing their own calves and sampled during early lactation, demonstrated considerably higher milk fat levels than that found in the early induced lactational milk of TOD and SLA. Although both of our orphan calves exhibited strongly positive growth curves for most of the nursing period, interpretation of the nutritive power of the suckled milk is complicated by our supplementation of both orphans with a milk formula and/or force feeding of whole fish during much of their nursing phase. Supplementation itself could conceivably influence nursing pressures from the calf, impacting in turn the quantity and/or quality of lactation to the extent that our milk analyses might serve only as preliminary guides to the nutritive potential of induced lactational milk. Yet, should milk produced in the early stages of induced lactation be truly nutritionally impoverished (or produced in small amounts), some form of supplementation to calves placed with foster mothers may be necessary to sustain their growth. The orphan nursing TOD was never solely dependent on suckled milk since he accepted whole fish voluntarily immediately following withdrawal of his supplemental feeding. In contrast, the orphan nursing SLA did experience a 5-mo suckling interval between the termination of his supplemental feeding and his willful acceptance of whole fish. During this time he appeared to thrive even though body weight, while increasing over the first 1.5 mo of the 5-mo "nursing only" period, showed a decline on three subsequent measures during the second and fourth months (12–14 mo of age). However, even a decrease in body weight during this period may signal no unusual nutritive weakness in the induced lactational milk, given the suggestion by Peddemors *et al.* (1992) that nutritional support delivered through suckling may, even for intact cow/calf pairs, be insufficient to sustain calf growth after 6 mo of age in the absence of solid foods. A decline in energy from milk as the calf nears weaning is supported by observations on a captive *Tursiops* cow/calf pair by

Cockcroft and Ross (1990). SLA's calf was reweighed at 19.5 mo of age after he had been accepting solid foods voluntarily for 4 mo; at this time he demonstrated a recovery in his growth curve with a gain over all previous weights.

In summary, it appears feasible from our observations that pairing of non-lactating adult dolphin females with unrelated orphan calves can provide a management option in situations involving abandoned, orphaned, or otherwise isolated calves. (Dry, non-pregnant females are probably better choices as orphan foster mothers than pregnant or lactating individuals, given reports of injury to an orphan by a female nursing her own calf [Smolders 1988]). Such pairings may offer a degree of nutritional support while simultaneously contributing to the nurturing benefits associated with cow/calf interactions and companionship.

Finally, it is interesting to consider a possible role for induced lactation in free-ranging cetaceans. Previous studies have shown that old toothed-whale females (pilot whale, *Globicephala macrorhynchus*) may be lactating even ten years after their last parturition (Kasuya and Marsh 1984, Marsh and Kasuya 1984, 1986). This observation was interpreted as an extension of lactation for the last calf in reproductively senescent females. Our observations suggest another possibility—these older cows may have relactated in response to suckling from calves produced by other females. Close genetic relations among members of pilot whale herds (Amos *et al.* 1993) could make lactational adoptions or ancillary nutritional support particularly adaptive to kinship survival strategies.

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